D. Research Design and Methods

This investigation involves two phases, development of the device prototype and testing in an animal model. These phases will be conducted concurrently and not in sequence as much as possible. That is, the process will be iterative and heuristic. A number of prototypes will be developed until bench top performance tests demonstrate the ability to fully expand the ends of the stent while the center portion remains undilated or minimally expanded. Once this is satisfied, the stent prototype will be coated with PTFE and its implantation attempted, with a complete angiographic and hemodynamic evaluation. The next prototype will be modified to adapt to any shortcomings with the first one. We have estimated (see section D.3, "Sample Size Estimate") that we need 18 evaluable study animals to detect differences in treatment effect between the two proposed treatment groups, and have included two extra in consideration that the first prototype may not be perfect, as well as for other potential drop out. We estimate that we may develop up to a half dozen stents for benchtop testing.

D. 1 Technology Development Plan

There is no commercially available medical device, stent or stent-graft, that assumes a variable-diameter shape (personal communication, Dorothy Abel in the Office of Device Evaluation at the FDA (301) 443-8517; dorothy.abel@fda.hhs.gov), and there is nothing available that would be remotely suitable for the proposed application. The devices will be made relatively inexpensively.

Developing a stent-graft that will inflate asymmetrically will be an engineering challenge. Commercially available balloon-expandable stent-grafts are designed to inflate symmetrically, with little diameter difference between the central and end portions during inflation. To do this, the dilatation balloon length is used similar to the device length. If the balloon is considerably longer than the stent-graft, it tends to inflate the "shoulders" of the balloon (or the part of the balloon not covered by the device) first, then the ends of the stent-graft, and finally the middle of the stent-graft. This can result in separation of the graft or fabric, and "accordioning" of the graft material into the center of the stent. This is highly undesirable. In order to engineer a stent-graft to intentionally dilate at its ends while maintaing relative constriction of the middle, the balloon should ideally be sized appropriately for the stent, but the stent will need more resistance to inflation in its center compared with the ends. Finally, bonding of the graft or fabric to the stent framework will have to be strong.

The prototype stents will be produced by direct ultraviolet (UV) ablation of portions of hollow 316 stainless steel tubing. The first stents we produce will be made using the design parameters we developed in the preliminary studies. Figure 5 is an illustration of the expanded stent elements. The center portion of the stent will be more ridged because the struts will be wider and they will have thicker shoulders. We will test the expansion of these stents by expanding them with a conventional balloon catheter and measuring the expanded diameter of the stent at eleven locations along the length of the stent. This will be done at 25%, 50%, 75%, 100% and 125% of the designed expansion pressures. We will be looking for uniform and smooth expansion of the stent, indications of any damage to the expansion balloon, problems with balloon deflation, and problems with balloon extraction. These experiments will be done with the catheter in free air and with the catheter in a simulated pig artery. It is expected that these initial studies will provide technical data which will assist in the next iteration of the stent design. As in conventional stent production, post processing operations may be required to address specific problems such as sharp edges on the struts. The ablation method of stent production will have a much smaller effect on the metallurgy of the stent and we do not expect any post processing will be required to remove slag.

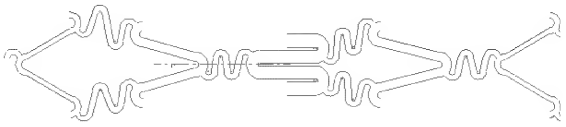

Figure 5. Illustration of expanded stent elements

The controlled ablation metal removal will allow some design freedom not available when using other production methods. One example of this is we can remove a portion of the wall material at the ends of the stent so that the ends will expand with a lower balloon pressure than the middle portion of the stent. Many conventional stents are built with struts having a square cross section (i.e. the width and thickness of the strut are nominally equal). In our first design we will reduce the width of the strut as we go from the middle of the stent to the ends. We expect that information from the testing of those stents will indicate how much thinner the struts will have to be made to meet the design requirements of an expanded diameter of 7mm at the ends and 2 mm at the center. It is our goal to design the stent so that the desired lumen diameter on deployment can be achieved with a single balloon design. The test procedure for the initial design will be updated to assure testing is complete and then the redesigned stents will be produced and tested.

Once the prototype stent framework has been developed, a "skin" will be applied to improve the ability to achieve a pressure gradient across the prosthesis, to reduce turbulence in the lumen and between the lumen and artery wall, and to reduce the likelihood of mural thrombus forming and embolizing downstream into bowel arteries. The "skin" will be expanded polytetrafluoroethylene (ePTFE), which is ideal for this application as it can be bonded securely by a proprietary patented process developed by Atrium Medical (Hudson, NH) while being low in bulk and preserving many of the advantages of a low-profile system. These include small access hole and trackability/deliverability to the artery of interest. The PTFE encapsulation is completed by taking a small ePTFE tube typically 1 mm in diameter and a little longer than twice the length of the stent. This tube is placed within the stent, with half of the excess length of material extending from either edge of the stent. Then the additional length of material is rolled over the outer edge of the stent to cover the outside of the stent. The outer layer is overlapped in this process. Once the PTFE is overlapped it is then sintered where the PTFE material binds together to form one piece. This process results in a bond that is very strong. A quote for coating 10 stents with ePTFE is included from Atrium Medical. After coating the stents with PTFE, they will be gas-sterilized. The coated stents will be retested by the above procedures to assure design parameters can be met with the coated stents and to assure the coating remains intact through the stent expansion process.

D.2 Prototype Testing: Animal Experiment

The animal experiment will be conducted in an 11,000 square foot animal facility at Rhode Island Hospital (Providence, RI) including an operating room, angiographic and fluoroscopic imaging equipment. The protocol will be submitted to the Rhode Island Hospital Animal Care and Use Committee, and Dr. Murphy will be the principal investigator. A collaboration letter from Luis Sousa, Ph.D., director of the animal laboratory at Rhode Island Hospital, is included in this application. All animal care will be provided per Rhode Island Hospital standard operating procedures and policy which are accordance with the U.S.D.A Animal Welfare Act Regulations done according to the ILAR *Guide for Care and Use of Laboratory Animals*. The animal facility has Full-Accreditation with the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC-International).

Stent-graft placements will be done in adult farm swine. Smaller animals, such as Yucatan or Hanford minipigs, will not be used due to their slower weight gain (we are examining differences in weight gain so the farm swine with their rapid change in weight are expected to show a difference more readily than slower growing pigs), and also since they are smaller the arteries may be smaller and technical placement of the stent-graft may be more difficult.

Farm swine are an excellent model of obesity[27] and will gain about a pound a day when fed liberally. They are also large enough to be treated with the prosthesis, in contrast to smaller animal models. Pig mesenteric arterial anatomy is similar to humans and has been well-characterized angiographically and pathologically[13]. In the pig, a celiac, "cranial" mesenteric artery, and "caudal" mesenteric arise from the abdominal aorta, with the "cranial" mesenteric artery supplying most of the small intestine to the ascending colon[13].

Pigs will be observed for dietary intake for 3 days prior to the procedure by weighing all dispensed food and subtracting that not consumed, and pigs will also be weighed pre-procedure. On the day of the procedure, animals will receive a dose of 325 mg aspirin orally, and then anesthesia will be induced with Telazol (tiletamine hydrochloride 50 mg/mL, zolazepam hydrochloride 50 mg/mL) 6 mg/kg and Rompum (23 mg/mL, (xylazine hydrochloride) by intramuscular (IM) injection and sodium pentothal to effect (20mg/kg) intravenously (IV), and then maintained under isoflurane gas anesthesia. Preoperatively, the animals also will be given Atropine 0.05 mg/kg, and buprenorophine (0.01 mg/kg intramuscularly (IM)) for pain. They will be placed supine in a sterile operating room environment, and the skin disinfected first clipping the hair around the surgical area in both groin regions followed with alternating scrubs with providone-iodine scrub and alcohol, and then with povidone-iodine solution. Access to one or both femoral arteries will be performed by cut down after assurance of adequate anesthesia. Once dissection of the common femoral artery is done, animals will be randomized to one of two treatment groups. For the control group, this will be the extent of the sham procedure, and the incisions will be closed.

For animals randomized to receive the stent-graft and flow restriction, once the common femoral arterie(s) are dissected free, vessel loops will be placed proximally and distally. Puncture of the common femoral artery(ies) will be done using an 18 G or 19G hollow-core needle, and a guide wire placed under fluoroscopic guidance into the upper abdominal aorta. A vascular sheath will be placed and connected to a flush of half normal saline at an infusion rate of 15-30 milliliters/hour. A flush catheter will be placed through the sheath and a flush abdominal aortogram obtained to map out the blood supply to the intestine. Selective catheterization of the gastroduodenal artery and superior ("cranial") mesenteric artery will be done with a shaped catheter and arteriography done of those circulations.

For active treatment group animals, occlusion of the gastroduodenal artery will be done proximally with coils placed transcatheter. The endovascular prosthesis will be placed in the superior ("cranial") mesenteric artery and deployed. The endoprosthesis is designed to inflate like a dumbbell, narrow in the middle but wide at its ends. The approximate lumen diameter on deployment will be 2 mm in the middle and 6 or 7 at the ends.

The sequence of events that include placement of the stent-graft in the cranial mesenteric arteries is as follows:

1. Stent-graft deployed in cranial mesenteric artery in its most constricted configuration with a lumen of 1-2 mm, having a dumbbell shape when looked at in longitudinal section
2. Stent-graft dilated until resting systolic pressure gradient is reduced to approximately 10-20 mm Hg

3. Vasodilation performed with tolazoline 25 mg intra-arterial in the cranial mesenteric artery to record change in resting systolic pressure gradient (this may be important to correlate with outcomes such as weight loss or adverse events as it correlates to the post-prandial pressure gradient)
4. If at any time during or after the procedure the pressure gradient is too high at rest and/or symptoms are too severe with eating or severe at rest, it can be completely reversed easily by dilation with an angioplasty balloon

Pressure measurements will be done distal and proximal to the prosthesis before and after injection of 25 mg of the alpha blocker tolazoline into the SMA. The resting gradient should be used as the endpoint for expansion of the stent graft—once the gradient is reduced to borderline hemodynamic significance (10-20 mm Hg systolic), dilation will stop. Tolazoline is a vasodilator and will augment any pressure gradient, and the gradient post-tolazoline may correlate to effectiveness at inducing ischemia after meals. The stent-graft lumen diameter will then be increased using angioplasty balloons if needed so that there is no or minimal gradient at rest (less than 10-20 mm Hg systolic). Final pressure gradients at rest and after tolazoline will be recorded to help monitor results vis a vis complications or weight loss, and to guide subsequent procedures. Subsequently, wounds will be closed using absorbable sutures beneath the skin (2-0 through 4-0 Vicryl, Ethicon/Johnson&Johnson, Warren, NJ) and staples for the skin, and animals will then emerge from anesthesia.

The animals will be monitored for two hours post-op to monitor behavior and level of activity to make sure that the animals have completely recovered from anesthesia. They then will be evaluated q 12 hours and given analgesia, buprenorphine 0.01mg/kg IM for 24 hrs for pain. They will receive aspirin 325 mg orally each day for platelet inhibition, anticoagulation is not done in clinical practice for humans with stents grafts and won't be done.

Pigs will be handled according to the National Science Foundation's Guide for the Care and Use of Laboratory Animals. Pigs will be observed for signs noted in the 1992 report, "Recognition and Alleviation of Pain and Distress in Laboratory Animals", and these findings will be noted as adverse events and reported in the safety section of the manuscript describing the study's results. Species-specific signs of pain and distress in pigs include hiding, increased attempts to avoid handling, increased squealing when approached or held, increased vocalization, poor gait, separation from the group, and unwillingness to move. Anorexia, which can be a sign of pain or distress in pigs, will be of poor discriminatory value in this experiment because of the goal of reducing food intake. If signs are observed consistent with severe pain or distress, such as hiding or unwillingness to move, repeat angiography and pressure measurements will be done. If the pressure gradient has increased since the procedure was done, the stent-graft or artery may be dilated to reduce the gradient. If the artery is thrombosed, or if the revascularization attempt is unsuccessful and these signs of severe distress are not relieved within 24 hours, pigs will be euthanized and autopsy done to evaluate intestinal viability grossly.

The pigs will be closely monitored for 7 days for changes in activity, behavior, eating and watering habits. If the pig is found to not be eating or to be experiencing any discomfort, the veterinarian will be consulted and additional doses of buprenorphine may be administered at their discretion. Euthanasia will be performed in an instance were the animal is found to be in significant distress or discomfort, continuing decreased food/water intake, or exhibiting lethargy despite treatment at the discretion of the veterinarian in consultation with the investigators.

Thereafter, the animals will be checked daily for any changes in activity, behavior, eating habits, urine or feces output. If any change is noted, appropriate evaluation will be done to determine if the animal is experiencing any pain or if there is an infection present (infection will be evaluated by visual examination of surgical sites for abnormal changes as well as temperature evaluation). They will be

monitored for dietary caloric intake for 3 day periods at one week and one month post-procedure. Weighers will be trained on the laboratory standard method of weighing, including weighing on a consistent time of day, and will be blinded to the treatment group. After 60 days, pigs will be weighed and sacrificed. Gross inspection of the bowel and mesenteric artery endoprosthesis done for descriptive purposes, including the presence of bowel ischemia, stricture, infarction, intraarterial thrombus or intimal hyperplasia.

D.3 Sample Size Estimate

Sample size was estimated based on the anticipated difference between groups at 60 days (t-test). It is anticipated that the use of repeated measures ANOVA at analysis will be more sensitive and so the calculated sample size represents a conservative estimate of that required. The following assumptions are made:

- The mean weight for the population of pigs at baseline is 160 lbs
- After one month of ad lib feeding, the control group mean weight is estimated conservatively at 170 lbs
- After one month of ad lib feeding, the treatment group mean weight will be 150 lbs
- The group standard deviation is 12 lbs
- The effect size, or standardized difference, ={(170-150)/12}=1.67

H_o: $\mu_{SH} = \mu_{ST}$
H_a: $\mu_{SH} \neq \mu_{ST}$

Where μ_{ST} is the primary endpoint estimate for the reducing stent-graft group and μ_{SH} is the primary endpoint estimate for the sham group at 60 days. The null hypothesis is that the sham group and stent group mean weight will not differ significantly at 60 days. The alternative hypothesis is that there will be a statistically significant difference in the mean weight of the stent group compared with the sham group at 60 days. Rejection of the null hypothesis will signify that the weight change between the two groups is significantly different. **Given the above, with an α of .05 and power of 90%, a sample size of 18 (9 per group) evaluable subjects is needed (EaST 2000 software, Cytel Corporation, Cambridge, MA).** We have inflated the sample size by two (10%) to account for study subject drop out such as for example due to technical failure of stent-graft implantation procedure and complications of either treatment, or drop out due to other unanticipated causes.

D.4 Statistical Analysis:

Data will be collected in laboratory notebooks and entered into a computer database (Access 2000, Microsoft, Redmond, WA) designed for this project. Data will be analyzed using StatView v.5.0.1 statistical software (SAS Institute, Cary, NC).

Primary endpoint:

Change in weight will be compared between treatment groups using repeated measures ANOVA with Group as a fixed effect.

Secondary endpoints:

1. Estimate of dietary caloric intake—Pigs will be fed *ad lib*, and excess amounts of feed will be left in the pen with each pig. Caloric intake will be calculated by measuring standard feed prior to filling the feed dispensing bin, and then at the end of a three day period measuring how much is left in the bin and in the pen, and therefore how much was consumed. Caloric equivalents will be calculated according to the feed manufacturer's information. The change in average caloric intake over the 3

day period pre-intervention compared with one week and one month later will be compared using a t-test.

2. Adverse events—Adverse events will be categorized as serious (death, bowel infarction, infection, hemorrhage) or nonserious (diarrhea, aversion to food, reduced activity), and will be monitored in the post-operative period as well as throughout the survival period. Species-specific signs of pain and distress in pigs include hiding, increased attempts to avoid handling, increased squealing when approached or held, increased vocalization, poor gait, separation from the group, and unwillingness to move will also be monitored as study endpoints as well as to help ensure humane treatment of the animals. These adverse events will be recorded so that risks of the proposed treatment can be understood, and any benefits in terms of weight loss be understood in the context of the risk of the treatment.

The quantitative milestones for this Technology Development Plan are as follows:

1. Laser cutting and ablation waste removal of the first prototype (first generation)
2. Bench tests of the first prototype(s) demonstrating successful controlled variable diameter stent inflation without stent asymmetry and easy balloon removal from the stent
3. Successful bonding of PTFE "skin" to prototype stent(s)
4. Bench tests of stent-graft (stent and bonded "skin") showing successful controlled variable diameter stent inflation without stent asymmetry and easy balloon removal from the stent
5. Re-design if necessary of stent-graft
6. Repeat bench tests
7. Successfully deploy second generation stent-graft(s) in pig mesenteric arteries
8. Modifications of system in response to first animal use
9. Deploy system/perform procedure in 10 pigs' mesenteric arteries and complete sham procedure in 10 pigs
10. Complete 60 day follow up of all pigs

The General Research Plan for Phase II is:

Depending on the success of Phase I, Phase II could consist of improving stent-graft design and more preliminary investigation in animals, or could be safety studies in human subjects. Once the results of Phase I are in, if the technology development went smoothly with compelling preliminary results (significant difference in weight loss between the treatment groups with few adverse events) we can request an investigational device exemption (IDE) from the U.S. Food and Drug Administration (FDA) to proceed to human subjects. This would most likely take the form of safety studies in a limited number of individuals. The design of such a study, data collected, and endpoints would largely be dictated by the FDA. Subsequent "Phase II" would then include clinical trials of first safety, then efficacy, and probably two clinical trials would be required for FDA approval to market the device.

Software Sharing Plan

We anticipate no software development as part of this project.

IGI Systems/Nonproprietary Interface

The imaging-guided intervention proposed in this study can be done using any fluoroscopic imaging equipment and does not require proprietary equipment or software to perform.

D.5 Key Personnel

Dr. Timothy Murphy is the Director of Quequechan Engineering, Inc., and an interventional radiologists practicing in Providence, Rhode Island. He is the Director of the Vascular Disease Research Center at Rhode Island Hospital, and a Professor, Research Track, of Diagnostic Imaging at Brown Medical School. He has over 13 years of experience as an interventional radiologist and performs procedures similar to those proposed as part of this experiment on a regular basis. He is a fellow of the Society of

Interventional Radiology, the American Heart Association, the Society of Vascular Medicine and Biology, and the American College of Radiology. He is the principal investigator of the CLEVER multicenter randomized clinical trial (Claudication: Exercise Vs. Endoluminal Revascularization, NHLBI R01 HL077221), and co-principal investigator of the CORAL Study (Cardiovascular Outcomes with Renal Atherosclerotic Lesions, NHLBI R01 HL071556-01). In addition to participating in prototype design and development, Dr. Murphy will perform the animal experiment as principal investigator, will collect and analyze data, and report study results.

Dr. Lamar Bullock received a Ph.D. in physics from Michigan State University and is an Adjunct Professor of Physics at University of Massachusetts-Dartmouth, and is the director of the photonics laboratory that includes the IX-300 Micro-Machining Laser. Dr. Bullock was formerly president of Boston Laser Technology, where he developed a unique excimer laser-based manufacturing process. He also designed and built laser imaging equipment to apply this process to volume manufacturing of a coronary stent for a company, Buckbee-Mears (St. Paul, MN), that was developing stents for Cordis/Johnson&Johnson (Warren, NJ), one of the largest manufacturers of vascular stents in the world. This project required close coordination with the stent designers to make optimal use of a new laser-based manufacturing process.